

SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1 *In utero* CRISPR-mediated NHEJ in the *R26^{mTmG/+}* mouse model. **(a)** Schematic representation of the *R26^{mTmG/+}* genome and gRNAs targeting the loxP sites 5' and 3' to the mT gene. CRISPR mediated excision of the mT gene and NHEJ results in a 545-bp PCR product. The non-edited PCR product is 2951-bp. **(b)** E16 *R26^{mTmG/+}* fetuses were injected with AAV9.SpCas9.mTmG, AAV9.Cre, Ad.SpCas9.mTmG, or Ad.Cre, and genomic DNA was harvested from liver, heart, lung, and brain at DOL1 for PCR analysis of mTmG editing. Representative of 4 replicate mice per experimental and control groups. **(c)** DOL1 liver genomic DNA of AAV9.SpCas9.mTmG, AAV9.Cre, Ad.SpCas9.mTmG, or Ad.Cre injected fetuses was analyzed by Sanger sequencing and demonstrates the expected sequence and indels following excision of the mT gene and NHEJ. Representative of 2 replicate mice per experimental and control groups. The **(d-i)** heart, **(j-o)** liver, **(p-u)** lung, and **(v-aa)** brain of non-injected *R26^{mTmG/+}* fetuses **(d,e,j,k,p,q,v,w)** and *R26^{mTmG/+}* fetuses injected with AAV9.SpCas9.mTmG **(f,g,l,m,r,s,x,y)** or Ad.SpCas9.mTmG **(h,I,n,o,t,u,z,aa)** were analyzed at DOL1 by fluorescent stereomicroscope **(d-aa)** for GFP expression and by immunofluorescence **(bb-ee)** for GFP and TdTomato expression (GFP, green; TdTomato, red; DAPI, blue). Representative of 4 replicate mice per experimental and control groups **(d-ee)**. Scale bars = 1mm **(d-aa)** and 100μm **(bb-ee)**. DOL, day of life; Li, liver; H, heart; Lu, lung; B, brain; NHEJ, nonhomologous end-joining.

Supplementary Figure 2 Organ targeting following *in utero* intravenous Ad.GFP injection. E16 Balb/c fetuses were injected via the vitelline vein with Ad.GFP. **(a, b)**, H&E; **b**, GFP immunofluorescence; **c**, GFP immunoperoxidase; **10×**) Histologic analysis of DOL1 livers demonstrated GFP expression. **(d, e)**, H&E; **e**, GFP immunofluorescence; **f**, GFP

immunoperoxidase; 10 \times) Livers of 3-month-old mice continue to demonstrate GFP expression but at lower levels than DOL1 mice. (g-z) In addition to liver, other organs were assessed for GFP expression at DOL1. (g-k) Brain, (l-p) heart, (q-u) lung, and (v-w) kidney; (g,l,q,v) brightfield, (h,m,r,w) GFP FSM, (i,n,s,x) H&E, 20 \times , (j,o,t,y) GFP immunofluorescence, 20 \times , (k,p,u,z) GFP immunoperoxidase, 20 \times . Representative of 3 replicate mice (a-z). Scale bars = 100 μ m (a-f, i-k, n-p, s-u, x-z) and 1mm (g,h,l,m,q,r,v,w). FSM, fluorescent stereomicroscope; H&E, hematoxylin and eosin.

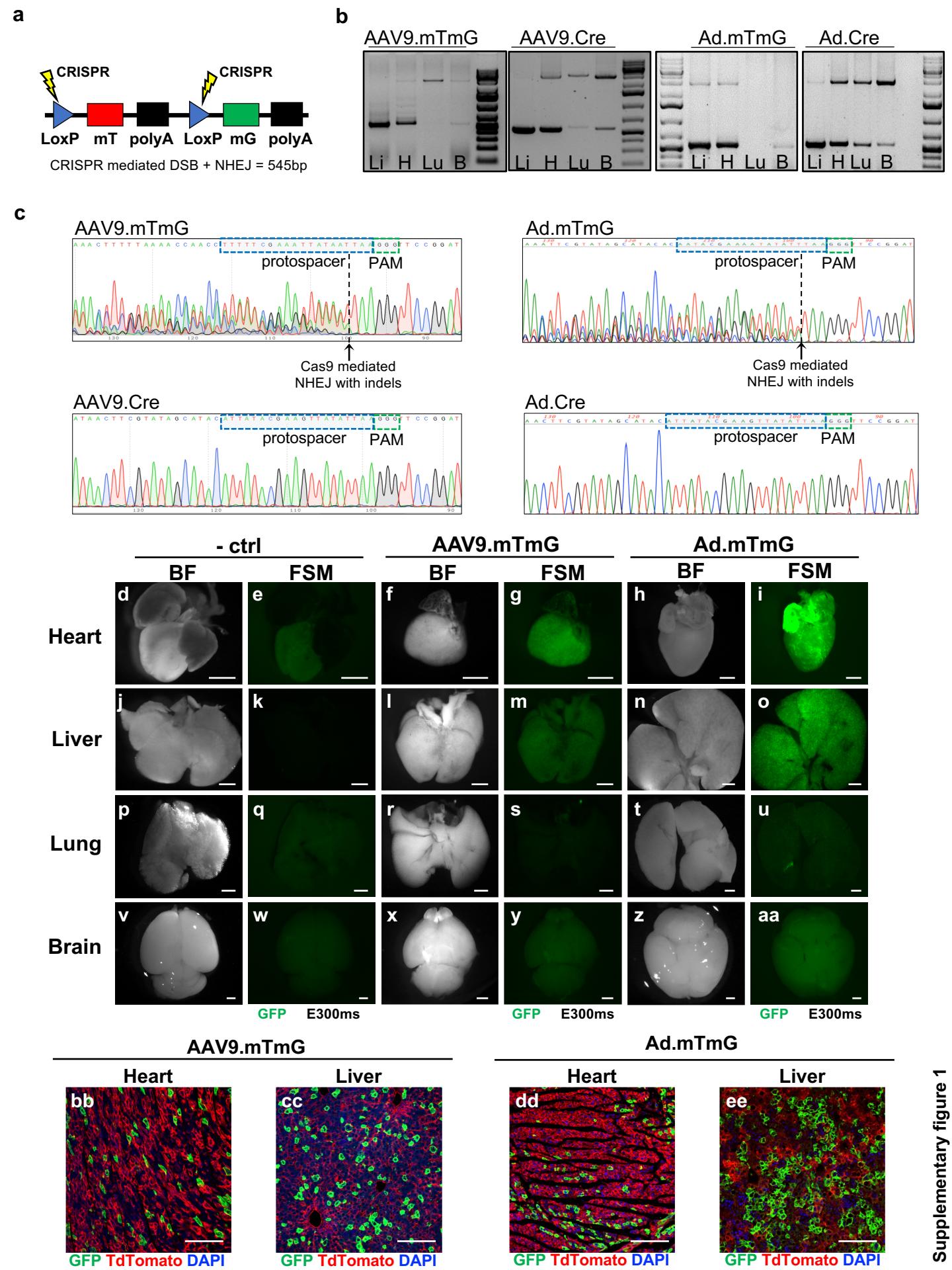
Supplementary Figure 3 *In vitro* *Hpd* gRNA screening and *in utero* base editing of *Hpd* in wild-type mice. (a) Tyrosine catabolic pathway highlighting the HPD enzyme, the target for base editing. (b, c) The *Hpd* sequence was screened for glutamine and tryptophan residues within the BE3 PAM base-editing window and thus amenable to conversion to stop codons via a C \rightarrow T change (sense strand) or G \rightarrow A change (antisense strand). Eight targets were identified. Blue bases fall within the BE3 window. Bolded underlined bases indicate the target codon. (d) On-target *Hpd* base editing was assessed by Surveyor assays; positive activity indicated by a “+” in (c). (e) Experimental scheme for *in utero* *Hpd* editing in wild-type mice. (f) Surveyor assays for *Hpd* on-target editing, liver genomic DNA, N=7 BE3.*Hpd* recipients, N=1 ctrl. (g) Sanger sequencing of liver genomic DNA from a mouse injected at E16 with Ad.BE3.*Hpd*. Representative of 3 replicate mice. (h) Fraction of on-target *Hpd* base-edited alleles, NGS of liver genomic DNA, N=7 BE3.*Hpd* recipients, N=1 ctrl, measure of centre = mean. (i) Surveyor assay, genomic DNA from other organs of prenatal Ad.BE3.*Hpd* recipients. N=3 BE3.*Hpd* recipients. Negative ctrl = non-injected Balb/c 2-week-old liver genomic DNA. (j) qRT-PCR to assess BE3 expression in indicated organs of prenatal Ad.BE3.*Hpd* recipients at DOL1 and non-

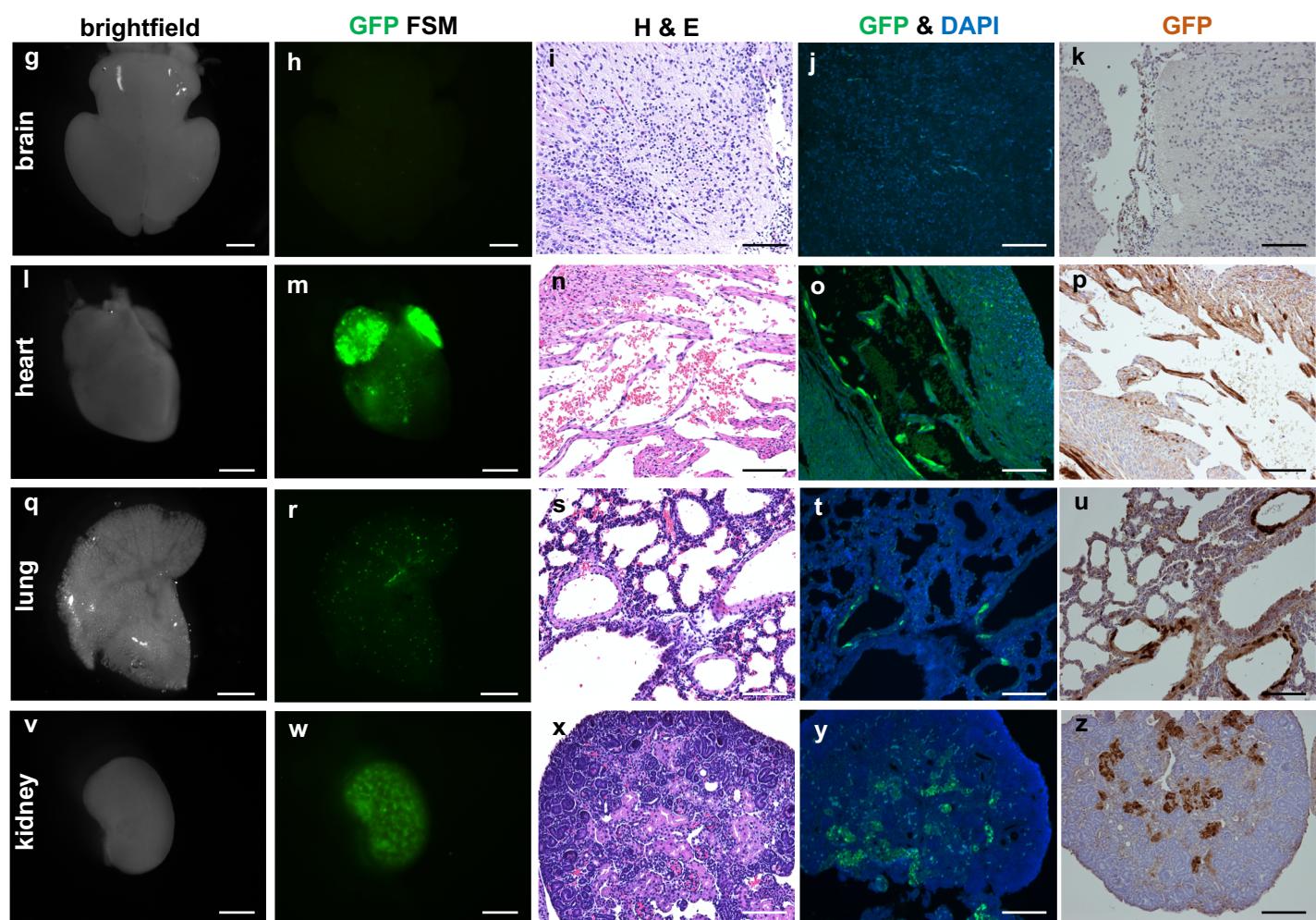
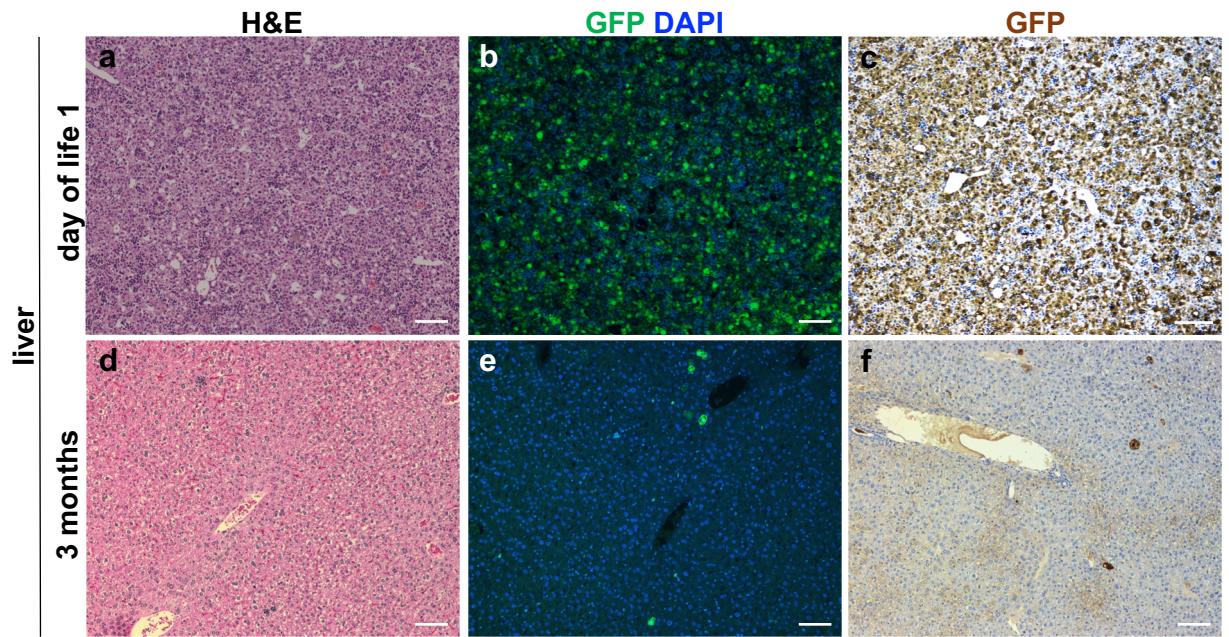
injected, age-matched controls; N=2 noninjected Balb/c, N=2 BE3.*Hpd* recipients; measure of centre = mean. **(k)** Liver (40 \times), heart (40 \times), lung (20 \times), and brain (20 \times) of mice prenatally injected with Ad.BE3.*Hpd* and non-injected controls were stained for SpCas9 to assess for BE3 expression. Representative of 3 replicate mice per group. **(l,m)** Livers from mice prenatally injected with Ad.BE3.*Hpd* (N=6) and non-injected (N=1) mice harvested at 2 weeks of age and stained for HPD. Percentages of HPD-negative cells were determined in ~100,000-300,000 hepatocytes per sample, measure of centre = mean. Scale bars = 100 μ m (k,l). BE3, base editor; FAH, fumarylacetoacetate hydrolase; HPD, hydroxyphenylpyruvate dioxygenase; IHC, immunohistochemistry; DOL, day of life; arrows, Surveyor cleavage products.

Supplementary Figure 4 Liver function and histology of *Fah*^{-/-} mice following prenatal Ad.BE3.*Hpd* injection. **(a)** Plasma samples from mice injected prenatally with Ad.BE3.*Hpd* (N=8) or Ad.BE3.Null (N=8), or non-injected mice maintained on NTBC (N=8), were assessed for ALT, AST, and total bilirubin levels at 3 months of age. Measure of centre = mean. **(b)** DOL1 livers from prenatal Ad.BE3.*Hpd* recipients and non-injected control mice were assessed by immunohistochemistry for HPD staining. Representative livers are shown. **(c)** H&E staining of livers (20 \times) at 1 month of age from non-injected Balb/c mice, *Fah*^{-/-} mice prenatally injected with Ad.BE3.*Hpd* and taken off NTBC at DOL1, non-injected *Fah*^{-/-} mice maintained on NTBC, and *Fah*^{-/-} mice prenatally injected with Ad.BE3.Null and taken off NTBC at DOL1. Samples from *Fah*^{-/-} mice prenatally injected with Ad.BE3.Null were harvested at DOL17 secondary to the inability of these mice to survive to 1 month of age. Representative of 5 replicate mice. Scale bars = 1mm (b, left panels) and 100 μ m (b, right panels and c). BE3, base editor; HPD, hydroxyphenylpyruvate dioxygenase; NTBC, 2-(2-nitro-4-trifluoro-methylbenzyol)-1,3

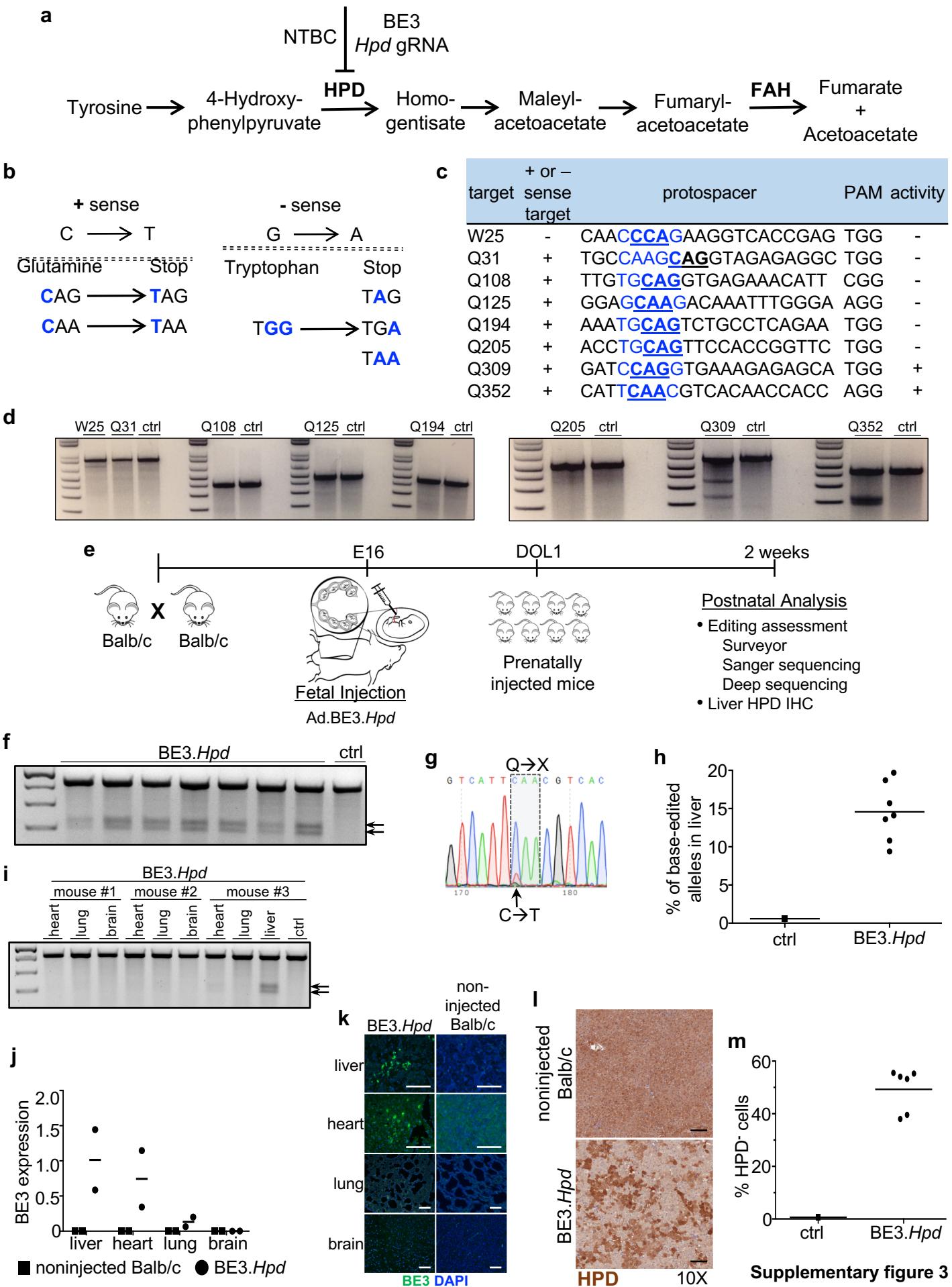
cyclohexanedione; ALT, alanine aminotransferase; AST, aspartate aminotransferase; FAH, fumarylacetoacetate hydrolase; DOL, day of life. Statistical analysis performed with Kruskal-Wallis test.

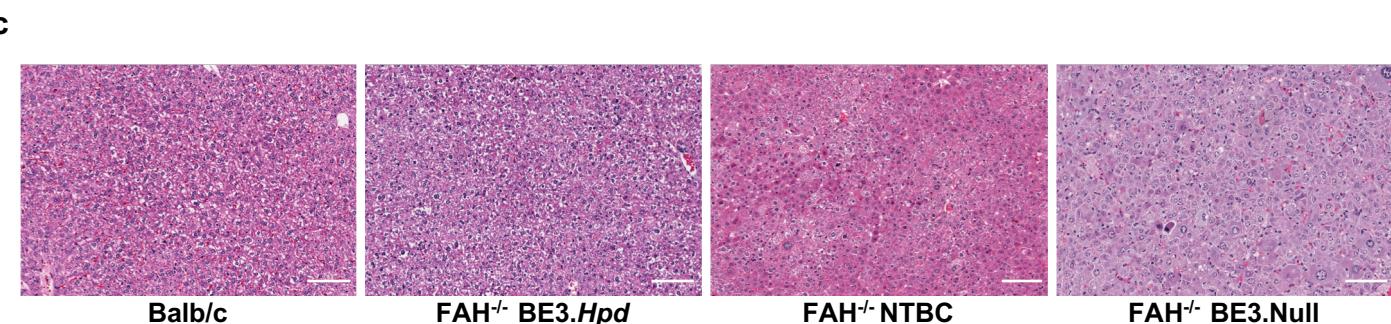
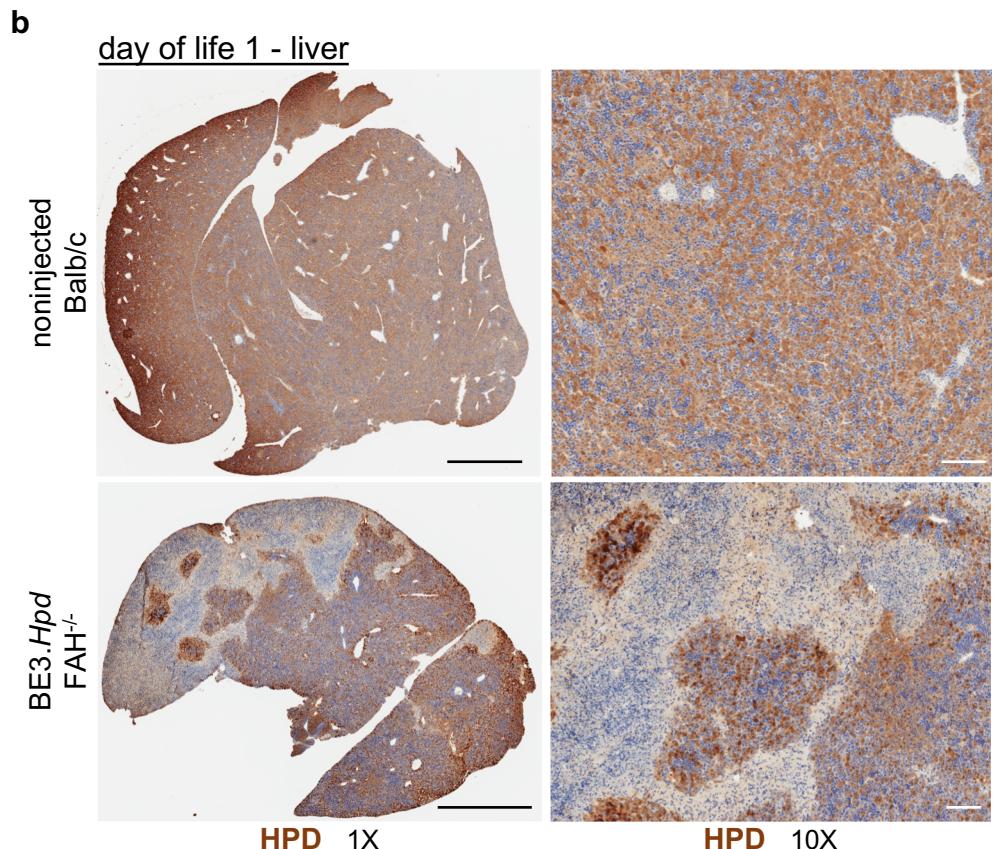
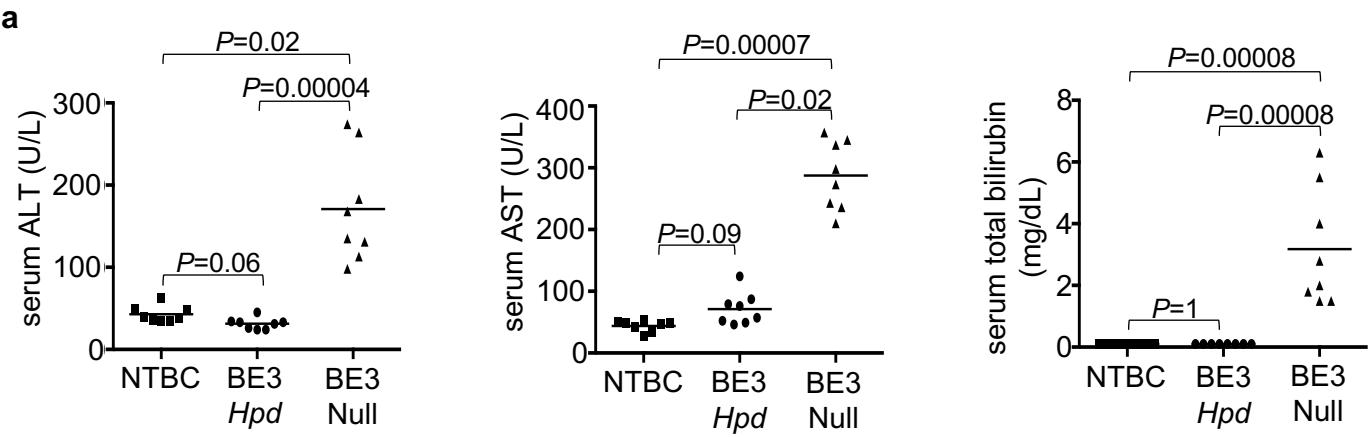
Supplementary Video 1 *In utero* vitelline vein injection of an E16 fetus with dilute trypan blue.





Supplementary figure 2





Supplementary figure 4

	target	protospacer	PAM
W25	CAA <u>CCCA</u> G AAGGTACCGAG	TGG	
Q31	TGC <u>CAAGC</u> AGG TAGAGAGGGC	TGG	
Q108	TTG <u>TGCAG</u> GTGAGAACATT	CGG	
Q125	GGAG <u>GCAAG</u> ACAAAATTGGGA	AGG	
Q194	AAA <u>TGCAG</u> TCTGCCCTCAGAA	TGG	
Q205	ACCT <u>TGCAG</u> TTCCACCGGTTTC	TGG	
Q309	GAT <u>CCAGG</u> TGAAAGAGAGAGCA	TGG	
Q352	CATT <u>TCAAC</u> GTCACAACCACC	AGG	

Supplementary table 1 *Hpd* sites screened for editing via Surveyor assay *in vitro* in N2a cells

Viral vector	PFU titer (IFU / mL)
Ad.BE3.Pcsk9	3.9×10^{10}
Ad.BE3.Hpd	4.8×10^{10}
Ad.BE3.Null	5.7×10^{10}
Ad.SpCas9.mTmG	1.2×10^{11}
Ad.GFP	1.0×10^{10}
Ad.Cre	1.0×10^{10}
AAV9.SpCas9.mTmG	1.0×10^{13} GC/mL
AAV9.Cre	3.9×10^{13} GC/mL

Supplementary table 2 Viral vector titers

target	primer		experiment
Hpd W25 & Q31	forward	TGAGTCCCATTCTCGGAGGT	Hpd <i>in vitro</i> screening
	reverse	CCACTGAAAAGCCCTTCCCT	
Hpd Q108	forward	CTGACATATGGATCAGGGCGT	Hpd <i>in vitro</i> screening
	reverse	AAGCTTCAGCGAGGCATTA	
Hpd Q125	forward	GGTAGCTAGAGGTGTTGGC	Hpd <i>in vitro</i> screening
	reverse	CAGACACCACCCCCCTTTCA	
Hpd Q194	forward	GCTGTTCAGTTAACCACGGC	Hpd <i>in vitro</i> screening
	reverse	GCCACCAAACCTGATGACCT	
Hpd Q205	forward	GAGGATCCTGTGTAACGGGTG	Hpd <i>in vitro</i> screening
	reverse	CCCTGCGGCTAATAAACCAGA	
Hpd Q309	forward	AATGTCACTCCGGCTTCTGT	Hpd <i>in vitro</i> screening
	reverse	GCATACTTGAAGGCTGTGCC	
Hpd Q352	forward	CTTGTTGGTGCAGTAGCCT	Hpd <i>in vitro</i> screening & <i>in vivo</i> studies
	reverse	CATGTGTGGATGGGGCTTA	
Pcsk9 W159	forward	TTCAGGGGCTGGAGTTACGG	Pcsk9 <i>in vivo</i> studies
	reverse	AGAGAGCACAGAGAGGTCAGT	
mTmG	forward	AAATCTGTGCGGAGCCGAAATC	mTmG PCR & Sanger sequence analysis
	reverse	CCTGTCCGTCGCTTGGAAAG	

Supplementary table 3 Primers used for Surveyor assays, mTmG PCR, and Sanger sequencing

Protospacer and PAM		Location	MIT off-target score
Pcsk9			
CAGGTTCCATGGGATGCTCT	GGG	Exon:Pcsk9	
CGCGGTCTATGGGATGCTCT	GGG	Intergenic:Snx8-Eif3b	1.35
CAGTTTCCATGGGGTGCTCT	TGG	Intron:Spata5	1.29
TGTTTCCATGGGATGCTCT	TGG	Intron:Ephb1	1.29
CAAGCTCTAAGGGATGCTCT	GGG	Intron:Cnnm1	1.24
CAGGATGGAGGGGATGCTCT	TGG	Exon:Cd5	0.82
CCGCTTCCCAGGGGATGCTCT	TGG	Exon:Mansc4	0.77
CAGGTTTATGGTATGCTCT	AGG	Intron:Shisa9	0.67
GAAGTTTCATTGGATGCTCT	GGG	Exon:Gpr165	0.53
CTGGTTCCATGGGAGGCTCA	AGG	Exon:Mtgl-Sprn	0.40
Hpd			
CATTCAACGTCACAACCACC	AGG	Exon:Hpd	
CATTCAACCTCACAAACCACA	AGG	Intergenic:Cbln2_Gm5096	2.37
CGGTTAACGGTCACAACCACC	TGG	Intergenic:B930078G14Rik=Fam155a	1.35
GGAGCAACGTCACAACCACC	AGG	Intron:Shank2	1.29
TATTCAAACCAACCACC	TGG	Intron:Lrrc4c	0.81
ATTTCAAAGTCACAACCACA	GGG	Intergenic:5730420D15Rik-Mrp142	0.7
AATTCAAGGTACAACCCCCC	AGG	Intergenic:Gm2574-Gm24728	0.66
CATAAAATGTCACAACCACA	TGG	Intron:Epha5	0.66
CCTTCACCGTCACAAACACC	TGG	Exon:Lpin1	0.37
CATACAAACATCACAAACCATC	AGG	Exon:Agbl5	0.61
CTTCATCAGCACAAACCACC	TGG	Exon:A4galt	0.52

Supplementary table 4 Off-target sites for *Pcsk9* and *Hpd*

target	forward primer	reverse primer
Pcsk9		
Pcsk9	AAGACTTTGTGAAGGCTGGGG	CTTCCTCTGTCTGGGCCAT
Spata5	TCAGTCATGTAACCTCCCCCA	AGAACAGATTGCAAGCCATGAA
Ephb1	CATGACCAGTGACCAGTGTG	GGAGACCTTCTGCCAGTTG
Cd5	AAGGAGAACTCCCTAGCACCA	GGAAGTGGCAGCACTCAA
Gpr165	ATTGTTCAGCAACCTGGGGAA	TTCCCATGAAATTCTAGGAGACC
Mansc4	GCTGGCTAGCATGGTCAT	TGGCCTTGTGCTGTGAAACTA
Shisa9	CCATCCCCTGACCAGATAGC	CCTTCCCACACAGGGTTTT
Mtg1/Sprn	AATGCTGACCGCCAAGAAGA	GGCGTCCTGGATGGTTATT
Snx8-Eif3b	AAGGAAGCCTCCCCTTGTTTC	TCTGAACCTTCCTGATGCTCC
Cnnm1	GGGGTGAGCTGTCCATGTG	CTGGGCTTGACACAGATTGG
Hpd		
Hpd	CCTTCCTTAACAGAGCCACT	TGGGTAAGATTCGCAGGCA
Cbln2	ACCTCTTGTGTCTAAGGGC	AGTCTGATTCAAATAGGAGAATGTC
B9300	CATTCCACACAACGCATTCC	ATGAACCAAACCCCTCAGCCT
Shank2	CATTGGTGGTGTCAAGCCTC	ACACCCAGCACAAGAGTTGA
Lrrc4c	GTTAGCCAAGGTTGTACCCCCA	GCTCAGGGTCTTCGCCTAA
5730	TTGGCCAGCTAGAAAGCAGA	CACATTCTGACGAAACCGC
Gm2574	AGTTCCGGATGTCTGAAGGGA	CTTATGCCCTGCATTCTGAT
Epha5	CATTATAAGGTACAATCAACGACA	GGGCCATGCTACAGCAGTT
Lpin1	TCGTTGAAACGGAGTGCTGA	GGCTGCCGGATGACAATGAT
Agbl5	AAACAGTGCTAACAGTGACGC	CCTGGAAGTCAAAGTGGGCT
A4galt	CTCAGAAGAGAGATGCCGAGG	AGCTGGCCTTCCTAGAGTC

Supplementary table 5 Primer sequences for next generation sequencing for *Pcsk9* and *Hpd* on- and off- target sites